The quest for the best cell factory for recombinant protein production: *Yarrowia lipolytica* vs *Pichia pastoris*

Marie Vandermies, Chrispian Theron, Patrick Fickers

Microbial Processes and Interactions, TERRA Teaching and Research Centre, University of Liège - Gembloux Agro-Bio Tech, Belgium
marie.vandermies@doct.uliege.be; pfickers@uliege.be

CONTEXT & MAIN RESULTS

- Recombinant protein (rProt) production is of increasing importance in the field of industrial biotechnology, and is notably performed through non-conventional yeasts such as *Y. lipolytica* and *P. pastoris* (*Komagataella phaffii*). Many studies report on the efficiency of these two yeasts for rProt production, but no direct comparison has been established on basis of the same protein.
- Here, the industrial lipase CalB from *Candida antarctica* served as a reference protein to compare the rProt production capacity of *Y. lipolytica* and *P. pastoris* at bioreactor scale.
- *Y. lipolytica* performances were far superior in terms of cell growth and extracellular lipase activity, despite *P. pastoris* showed a significantly higher level of CalB gene expression.
- Neither of CalB inactivation, codon usage bias, or CalB processing and secretion could be incriminated. The answer lies on the side of the unfolded protein response (UPR) and the endoplasmic reticulum-associated degradation (ERAD) observed in *P. pastoris*.

COMPARISON IN BIOREACTOR

EXPERIMENTAL CONDITIONS

- Pre-Pro-CalB sequence for secretion
- Inducible promoters
- Rich medium supplemented with:
  - glycerol (carbon source) and erythritol (inducer) for *Y. lipolytica*
  - sorbitol (carbon source) and methanol (carbon source & inducer) for *P. pastoris*
- Dasgip bioreactors, 2 repetitions, 72-h culture

CELL GROWTH & CARBON UPTAKE

Table 1: Dynamics of growth and carbon uptake rate during cultures in bioreactor.

	Y. lipolytica		*P. pastoris*
Growth rate (h^{-1})	**0.31 ± 0.06**	>	0.27 ± 0.08
Final biomass ($gDCW.L^{-1}$)	**10.1 ± 0.2**	>	4.8 ± 0.1
$Y_{X/S}$ ($g_{DCW}.molC^{-1}$)	**16.8**	>	8

➡ More than 2x more final biomass for *Y. lipolytica*

CALB EXPRESSION AND PRODUCTION

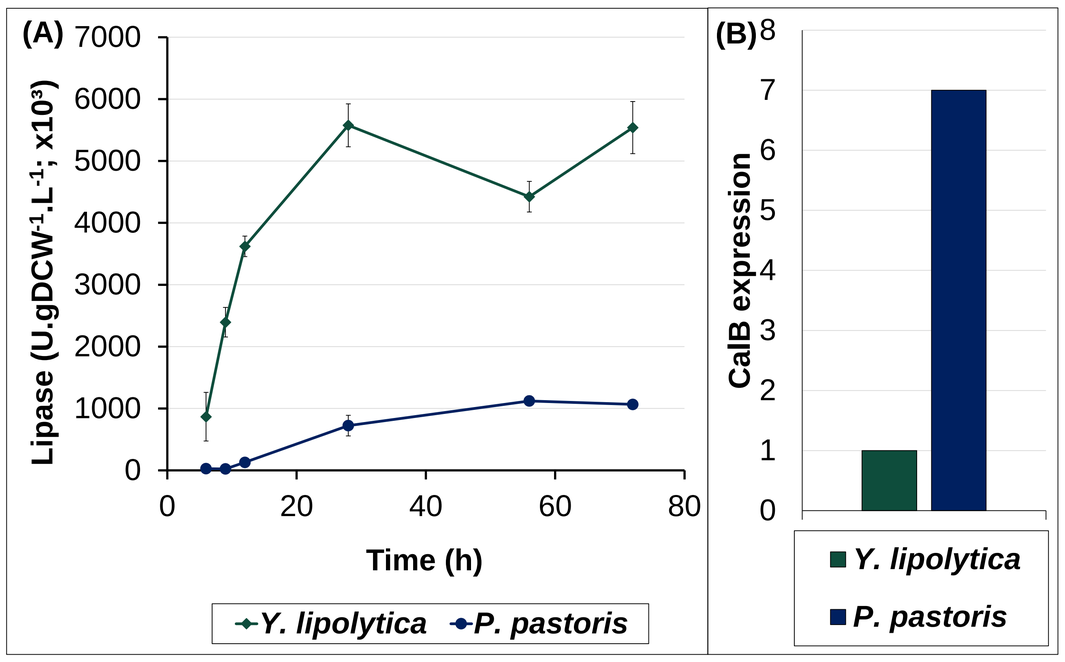

Fig. 1: (A) Specific lipase activity throughout cultures in bioreactor.
(B) CalB gene expression at the end of the exponential growth phase.

➡ More than 5x more specific lipase activity for *Y. lipolytica*
➡ 7x more CalB expression for *P. pastoris*

INVESTIGATION OF THE DIFFERENCE

CALB LIPASE INACTIVATION? X
No inhibitory compound in *P. pastoris* culture supernatant

CODON BIAS? X
No rare codon in CalB sequence for *Y. lipolytica* or *P. pastoris*

CALB PROCESSING DEFICIENCY? X
No difference in lipase activity between Pro-CalB and CalB for *P. pastoris*

CALB SECRETION SATURATION? X
No accumulation of GFP or GFP-CalB in *P. pastoris* cells

UPR & ERAD? V
- Upregulation of *HAC1* (UPR marker gene) and *DOA1* (ERAD marker gene) in *P. pastoris* cells producing CalB or GFP
- Higher fluorescence with the addition of MG-132 (ERAD inhibition) for *P. pastoris* cells producing GFP

CONCLUSIONS

- Higher expression levels do not systematically lead to higher rProt yields.
- Other factors may influence rProt production (in this case, CalB degradation within *P. pastoris* cells in relation to UPR and ERAD).
- In the present study, the proposed *Y. lipolytica* system appears superior to the proposed *P. pastoris* system regarding CalB production (in terms of cell growth and specific lipase activity).
- Further investigation on basis of other types of rProt shall be conducted, to determine if the difference observed between *Y. lipolytica* and *P. pastoris* is a general trend, or if the host cell factory shall be chosen according to the protein of interest.

ACKNOWLEDGMENTS

M. Vandermies and C. Theron received fellowships from FRIA (FNRS, Belgium) and BEWARE (DGEE, Belgium), respectively.